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***Helicobacter pylori* Infection and Undiagnosed Dyspepsia in Dyspeptic Populations Under 45 of Age Tested by ELISA, Urease Breath Test and Helicotest**

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1. Introduction

1.1 *Helicobacter pylori* infection

One-half of the world's population is infected with *Helicobacter pylori* (*H. pylori*), a gram negative bacterium which is responsible for various major upper digestive tract diseases. *H. pylori* is one of the most common bacterial pathogens in humans, who are the only known host of *H. pylori*. The human stomach is considered the reservoir of this bacteria.

H. pylori has been cultured from saliva, dental plaque, vomitus, and diarrheal stool demonstrating that the bacterium is potentially transmissible by these routes. The main route of transmission is not yet clearly understood.

H. pylori transmission is believed to be mainly familial, and there is epidemiological evidence that shows that the infection spreads via person-to-person contact (14). Moreover, potential reservoirs of bacterium are thought to be animals who are in close contacts with humans: cats, dogs, pigs, and birds. There is also the hypothesis that the most predominant mode of transmission is mother-to-child via contact with regurgitated gastric juice from the mother's mouth. The most common accepted routes of transmission are fecal-oral in developing countries, and gastro-oral route in developed countries. There are many risk factors for *H. pylori* infection including: overpopulation/congested houses, family sizes, unsafe sources of water, and low socioeconomic status (17).

The common risk factors for *H. pylori* infection is:

- crowding
- low level of personal hygiene
- low family income
- unclean drinking water
- lack of toilet facilities during childhood
- low educational level
- previous gastrointestinal endoscopy

H. pylori infection is recognized as a worldwide problem as it causes chronic gastritis, peptic ulcer disease, and Mucosa-Associated Lymphoid Tissue lymphoma. *H. pylori* is also a major risk factor for gastric cancer. The global burden of gastric cancer is considerable but varies in different countries, with more than 70% of cases occurring in developing countries especially in Eastern Asia. In 2008, it was estimated that there would be just under one million new cases of stomach cancer (Tab.1) Stomach cancer accounts for 7.8% of the all total cancers worldwide, and it is currently the fourth most common malignancy in the world, behind lung cancer, breast cancer, and colon cancer. Stomach cancer is more common in men (640 000) than in women (348 000), and half of the world’s stomach cancers occur in Eastern Asia and China. The highest mortality rates are estimated in Eastern Asia (28.1 per 100,000 in men, 13.0 per 100,000 in women), and the lowest in Northern America (2.8 and 1.5, respectively). High mortality rates are present in both sexes in Europe, and in Central and South America.

Estimated numbers (thousands)	Men		Women		Both sexes	
	Cases	Deaths	Cases	Deaths	Cases	Deaths
World	640	463	348	273	988	736
More developed regions	173	110	101	70	274	180
Less developed regions	467	353	246	202	713	55
China	315	231	148	121	463	352
European Union (EU-27)	50	37	32	24	82	61

Globocan 2008

Table 1. Stomach Cancer Incidence and Mortality Worldwide

The prevalence of *H. pylori* ranges from <10-20% in the USA, 40% in Germany, and 30-40% in England to more than 70% in Eastern Europe or even up to 90% Asia and Africa. The percentile of infection is also higher in rural areas than in big cities (5).

There are differences in *H. pylori* prevalence between high and low-income countries because *H. pylori* infection is strongly related to economic conditions (17). *H. pylori* incidence also increases with age largely due to the birth cohort effects. The children are re-infected more frequently than adults and because the close contact between young children, especially among siblings and children under the age of 5. In developing countries infection occurs in the first years of life and increases successively involving almost 90% of the 50-year-olds. In developed countries it affects only a small percentage of children below 10 years of age and does not exceed 40% in adults. It has been suggested that treating infected children reduces the transmission of infection and ultimately reduces the gastric cancer in adults, but the role of potential *H. pylori* eradication for the prevention of gastric cancer is still unknown.

The effects of *H. pylori* infection on human gastric physiology are complex. Presence of the bacteria induces chronic inflammation via the release of chemokines and cytokines. *H. pylori* infection also stimulates the human immune system, causing T and B lymphocytes, along with neutrophils and monocytes to produce antibodies (Ig G and IgA) and cytokines (TNF

alpha, interleukin). This immune response is unable to eliminate the pathogen and leads to persistent gastric mucosal damage as more neutrophils, lymphocytes, and plasma cells are recruited to chronic inflammatory sites (Tab.2).

Activation of limphocyte	Stimulation of neutrophile and monocyte
<ul style="list-style-type: none">Lymphocyte B (↑Ig G, ↑IgA) in human blood	<ul style="list-style-type: none">↑Histamine
<ul style="list-style-type: none">Lymphocyte T (IL-2,IL-8, TNF alfa)	<ul style="list-style-type: none">↑ Interleukin (IL-1,IL-6, IL-8)
	<ul style="list-style-type: none">↑ TNF alfa
	<ul style="list-style-type: none">↑ Interferon
	<ul style="list-style-type: none">↑ Prostoglandin E2

Table 2. The human immune system response to *H. pylori* infection

The virulence of the bacteria depends on the presence of the cag pathogenicity island which is a 35-40 kb genomic fragment containing 29 genes. The cagA protein is a well-known virulence marker encoded by the cagA gene. The presence of this gene has been associated with both duodenal ulcers and gastric cancer. CagA is phosphorylated and binds to SHP-2 tyrosine phosphatase and induces the intracellular signaling processes.

Another virulence marker is 95-kd vacuolating cytotoxin VacA which is related to the cag pathogenicity island. The VacA toxin opens the membrane channels of gastric epithelial cells to give the bacteria nutrients, and the mitochondrial membrane releases cytochrome c to induce apoptosis.

The *H. pylori* infection can be diagnosed by invasive and noninvasive testing (Tab.3). "Test and treat strategy" everywhere in *H. pylori* infection is recommended for patients with uninvestigated persistent dyspepsia less than 45, 50 or 55 years depending on country's guidelines without any alarm features (24, 25, 26). The "test and treat strategy" is based on non-invasive testing of *H. pylori* infection.

<i>H. pylori</i> diagnostic tests	
Invasive	Non-invasive
Gastroscopy <ul style="list-style-type: none">standard videoendoscopyhigh magnifying endoscopychromoendoscopy	Serology <ul style="list-style-type: none">near patient tests (HelicoTest)ELISA
Rapid urease test (CLO test)	UBT test C13, C14
Histology (Giemsa staining)	HP stool antigen test (HPSA) <ul style="list-style-type: none">polyclonal antibody-based ELISAmonoclonal antibody -based ELISA
Microbiology Culture	Gastropanel <ul style="list-style-type: none"><i>H. pylori</i> antibodiesPepsinogen I, IIGastrin 17

Table 3. Diagnostic modalities of *H. pylori* infection

1.2 Functional dyspepsia

The detection of current *H. pylori* infection is becoming more important clinically, especially in young patients, because the eradication of infection is likely to affect the natural course of the disease and modify the risk of gastric cancer. Early detection and eradication of *H. pylori* in the population will decrease major upper tract organic diseases as well (13).

Strategies to improve the management of *H. pylori* infection in the dyspeptic population with upper gastrointestinal symptoms have been shown to reduce mortality in the population caused by *H. pylori* infection. It is good to note that there is still more research needed regarding serology tests in young dyspeptic patients.

The Maastricht III-2005 Consensus report recommended serology as an alternative option for countries with a high prevalence of *H. pylori* infection (19, 20). The two serology tests that are most often used are the ELISA test and the near patients tests. The serology tests have the lowest cost per correct diagnosis at low (30%), intermediate (60%), and high prevalence (90%) of *H. pylori* infection but their diagnostic accuracy is lower than other noninvasive tests. The Urea Breath Test (UBT) is more costly, more time consuming, and needs special preparation by the patient, such as fasting and cessation of antibiotics, proton pump inhibitors (PPI), and H₂ blockers. The doctors who care for undiagnosed dyspeptic patients need to be aware of the value of these serology tests in young patients.

2. Dyspepsia and gastrointestinal symptoms

Gastrointestinal symptoms are common, with up to one in three people in population-based studies reporting symptoms from the gastrointestinal tract (Tab.4). Research published in 2008 in Sweden has shown a prevalence of symptoms from upper and lower gastrointestinal tract in adult population study even up to 60%. Symptom flux over time provides primary care physicians with a significant workload.

Gastrointestinal symptoms	Pain modalities
1. Acid regurgitation	1. Aching
2. Belching	2. Butterflies
3. Burning feeling rising from the stomach up towards the neck	3. Burning sensation
4. Dysphagia	4. Cramp
5. Early satiety	5. Colic
6. Heartburn	6. Gripes
7. Nausea	7. Pain
8. Loss of appetite	8. Tenderness
9. Loss of weight	9. Twinge
10. Pain behind the breast bone	10. Stitch
11. Uncomfortable feeling of fullness	11. Sinking feeling
12. Vomiting (recurrent)	

Table 4. The 12 general upper gastrointestinal symptoms from Carlsson & Drossman. 11 abdominal discomfort or pain modalities.

Gastrointestinal symptoms have been grouped into several entities (Fig.2). Depending on the symptoms profile, dyspeptic symptoms ranged from 15-40% in the population, reflux symptoms 15-25%, and irritable bowel symptoms 10-20%. About 14% of all patients will have dyspeptic, reflux, and irritable bowel symptoms when they are presenting in the primary care setting.

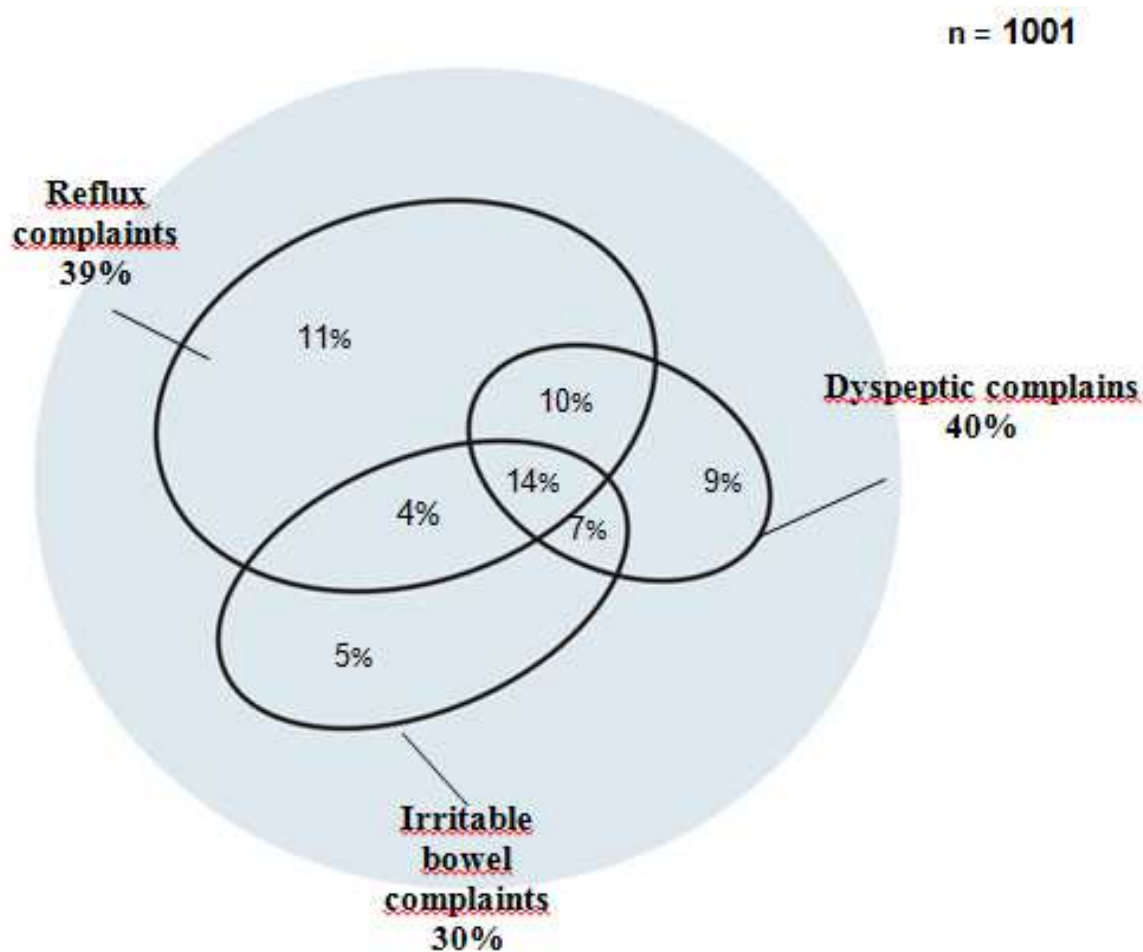


Fig. 2. The prevalence of reflux, dyspeptic and irritable bowel complaints in the general population.

Data was collected from two neighboring communities in Northern Sweden, Kalix and Haparanda, with 18,408 and 10,580 inhabitants, respectively (Kalixandra Study) has showed that most gastrointestinal complaints classified as reflux, dyspeptic, or irritable bowel complaints lasts over 6 month.

The short-term fluctuation of gastrointestinal symptoms in the general population is a fact (Fig.3). Reflux complaints are slightly more stable in comparison to the dyspeptic symptoms or irritable bowel complaints. During times of stress and emotional problems dyspeptic problems can be more severe and cause more problems for the patient, but only a fraction of sufferers will seek medical care (4). It is important to remember that troublesome gastrointestinal complaints remained present in approximately 90% of subjects in all symptoms groups if not diagnosed and treated.

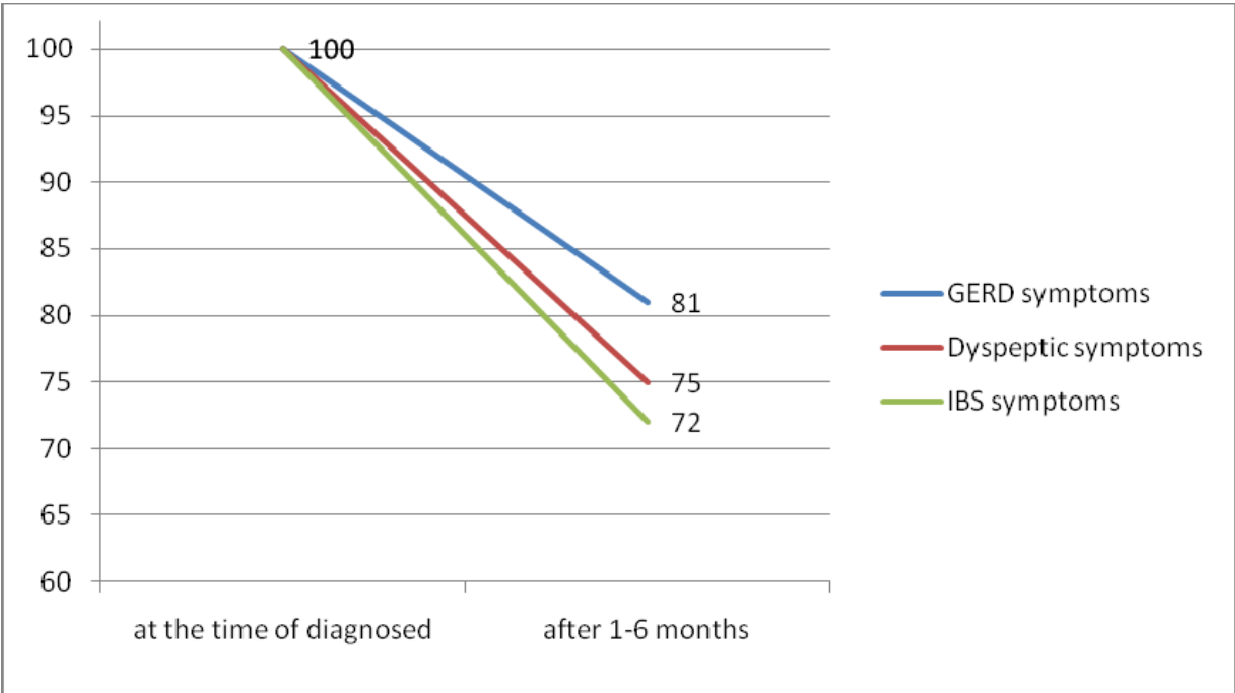


Fig. 3. Fluctuation of gastrointestinal complains in the short term from 4 weeks to 6 months.

3. Undiagnosed dyspepsia

Dyspepsia is very common and most patients will experience symptoms occasionally (Fig.4).

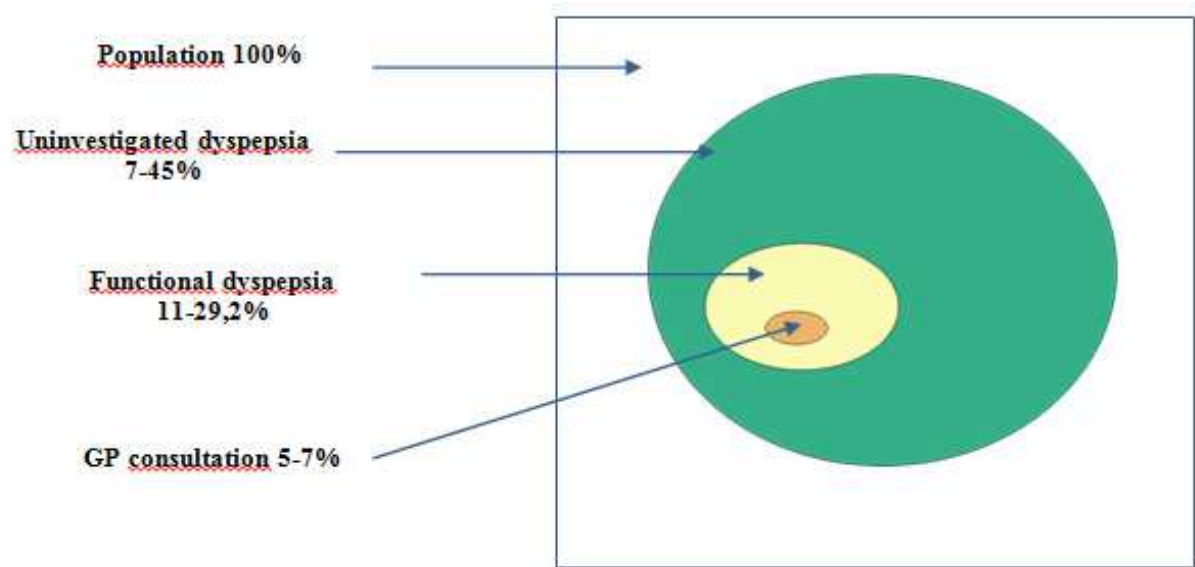


Fig. 4. Prevalence of dyspepsia in the community.

If the symptoms are chronic and recurrent medical management is needed. If the patients have symptoms occurring more than twice a week or lasting for over 4 weeks medical help is needed because of the major decrease in quality of life (2).

The life impairment and impact of undiagnosed dyspepsia on health-related quality of life (HRQOL) is significantly lower in dyspeptic patients than in healthy controls (0,85+/-0,17 vs 0,95+/-0,12) p<0,0001 (15).

Dyspepsia is diagnosed as a syndrome consisting of pain or discomfort centered in the upper abdomen (epigastric pain), burning, fullness, discomfort, early satiety, nausea, vomiting, and belching.

Many patients with undiagnosed dyspepsia are usually young and there is overlap of symptoms and findings in performed investigations regarding to most functional symptoms like Irritable Bowel Syndrome or Functional Heartburn (9).

If alarm symptoms occur in patients with undiagnosed dyspepsia, upper gastroscopy is needed (Tab.5).

• Signs and symptoms of upper gastrointestinal bleeding
• Unexplained anemia
• Unexplained weight loss
• Progressive dysphagia
• Recurrent vomiting
• Progression of symptoms
• Previous gastric surgery
• Family history of gastrointestinal cancer
• <45,50, or 55 years of age (depending on country)

Table 5. Alarm symptoms for uninvestigated dyspepsia and indications for upper gastrointestinal endoscopy.

The epidemiology of dyspepsia can influence of many factors such as: cultural differences in reporting of symptoms of dyspepsia, socio-economic status, cigarette smoking, *H. pylori* infection, use of non-steroidal anti-inflammatory drugs, and alcohol consumption (Tab.6).

• Main Risk Factors:	• Odds Ratio
• <i>H. pylori</i> infection	• OR 1,21 (CI;1,03-1,42)
• Cigarette smoking 20/ day	• OR 1,55 (CI;1,29-1,86)
• Unemployment	• OR 2,18 (CI2,86-2,56)
• Daily use of ASA/NSAID	• OR 2,33 (CL;1,72-3,15)
• Others: Alcohol consumption, social status, life style factors, level of education	

Table 6. Risk factors for dyspepsia in a general population a total 10 007 aged 40-64 years.

Research has shown that patients profiles and risk factors are important in the management of dyspepsia, and its role is increasing with the patients age. Common use of non-steroidal anti-inflammatory drugs, high prevalence of *H. pylori* infection in many countries, and the increasing prevalence of smoking in the young population make dyspepsia a very common ailment (31).

Initial management with prompt endoscopy slightly more effective (3,7,8) compared to the “test and treat” approach for inducing resolution of symptoms, but is not as cost effective as the “test and treat” strategy (Tab.7).

Outcomes	RRR (CI)
Presence of symptoms	5% (1 to 8) significant difference favours for endoscopy
	Standardised mean difference (95% CI)
Total dyspepsia symptoms score	-0,11 (-028 to 0,07)
	Weighted mean difference (CI)
Additional cost of prompt endoscopy (US dollars)	\$389 (276-502) significant differences favour "test and treat strategy"

Table 7. Initial management of dyspepsia with prompt endoscopy v “test and treat” strategy at 12 month.

There is an overlap of symptoms and finding structural abnormalities in upper endoscopy, however about 60% of all endoscopies performed will not find any structural changes in upper digestive track (Fig.5).

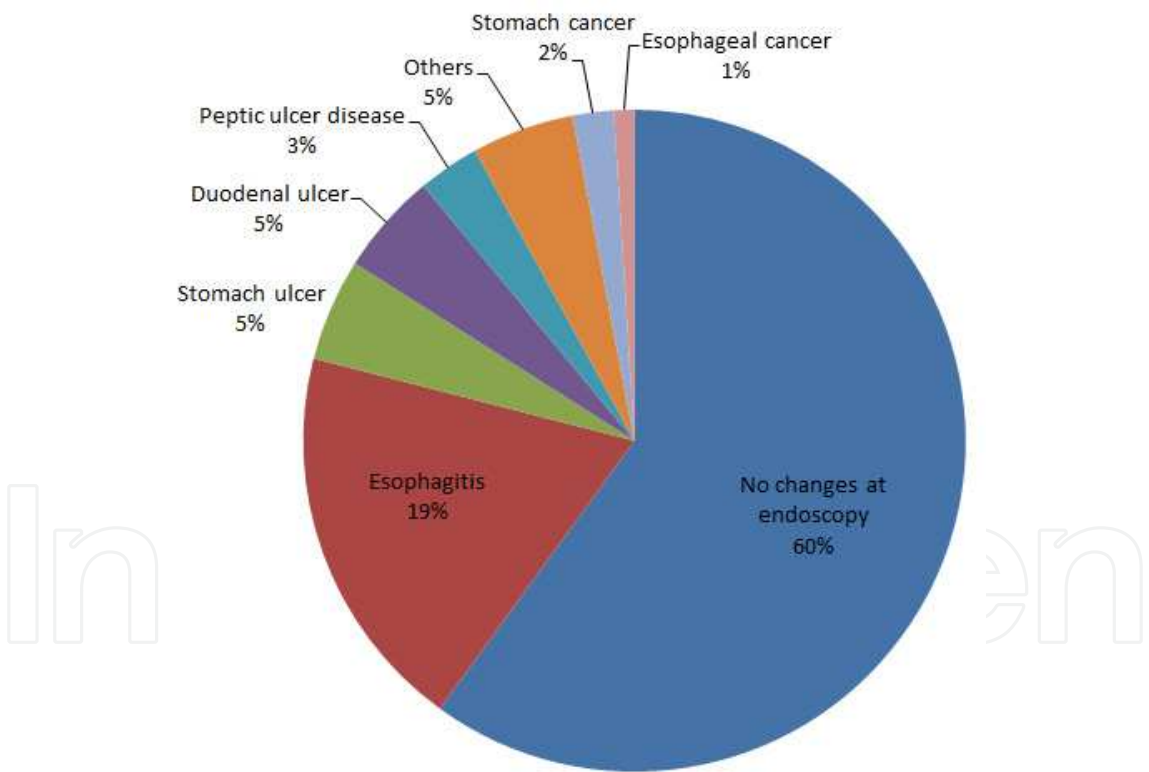


Fig. 5. Abnormal finding at upper digestive track during performing gastroscopy.

40% of all patients with dyspeptic complaints will have some small mucosal changes in upper gastrointestinal tract. Ford et al. provide the first meta-analysis of individual patients of 5 management trials. Their results strongly support the “test and treat” strategy in terms of cost effectiveness. The prevalence of organic dyspepsia as classified by endoscopy increased with the patient’s age (23). A proportion of patients have an underlying organic

cause for their symptoms such as peptic ulcer disease or reflux esophagitis. However, only 3% of people in the population-based study were found to have an organic disease diagnosed in the general health care system.

Peptic ulcer was found in only 3.9% of all subjects with upper abdominal symptoms in population based study by Bernersen et al. Aro et al. found that 4.1% of the subjects have peptic ulcers and minority of those with gastroesophageal reflux disease were found to have esophagitis on endoscopy which still is the most commonly found change on this procedures (29).

There is great debate in the medical community about whether gastrointestinal symptoms in the general population should be regarded as a disease or not. Many cases of epigastric pain are diagnosed as functional dyspepsia (FD) without any organic changes found in upper endoscopy. FD can be diagnosed if the symptoms are chronic and not caused by another organic, systemic, or metabolic disease. Many of those with dyspeptic symptoms will have functional dyspepsia, gastrointestinal motor abnormalities, or altered visceral sensation. FD can be considered as bio-psychological disorder causing a dysregulation of the brain-gut axis (1).

A meta-analysis of 9 trials evaluating a total of 2541 patients with FD found a modest but significant benefit to *H. pylori* eradication treatment at 12 months. According to the analysis the mean response rate to placebo at 1 year was 28% (range 7-51%) and the mean response rate to *H. pylori* eradication 36% (range 21-58%). Many researchers think that eradication *H. pylori* might be a cost-effective intervention for FD.

4. The aim of the study

1. The main aim of the study was to compare the accuracy of serology tests (ELISA, HelicoTest) with the benchmark UBT in the detection of *H. pylori* infection in young dyspeptic patients age 20-45 who presented with chronic dyspeptic symptoms over 6 months at the primary care setting.
2. The additional aims of the study determine whether a correlation existed between the level of Ig G antibodies and positive UBT test.

5. Material and methods

The study was conducted from 2004-2006 in the primary care setting in Cracow. Each of the enrolled patients underwent the following procedures:

- Detailed history
- Physical examination
- C13 Urease UBT test
- Serological ELISA-DPC test
- HelicoTest - a rapid test for quick detection of IgG antibodies
- Epidemiological questionnaire using the Glasgow Dyspepsia Severity Score (GDSS) which assesses dyspepsia symptoms (10).

The most frequent symptoms of dyspeptic patients were: epigastric pain, upper abdominal discomfort before and after a meal, and dysmotility-related symptoms (bloating, nausea,

belching, occasional heartburn). The study was approved by the Ethics Committee of the Jagiellonian University, and all participants gave written informed consent. The gold standard of diagnosis of *H. pylori* infection was based on a positive UBT test. Patients with alarm symptoms were not included into the study.

Exclusion criteria included: weight loss, anaemia, hematemesis, melena, abdominal tumor, use of non-steroidal anti-inflammatory drugs, use of antibiotics or ranitidine bismuth citrate four weeks prior to the investigation, use of PPI two weeks and H₂ blockers 48 hours prior to investigation.

Statistical analysis was carried out using the STATISTICA and SAS programs. The agreement of the tests was assessed by three criteria: the percentage of incompatible results, the value of the kappa coefficient, and McNemara’s test for related dichotomous variables. P<0.05 was considered statistically significant. Distinguishing a positive or a negative *H. pylori* result was objective in UBT and positive ELISA was considered at the level of 1.00 U/ml.

6. Results

The study group consisted of 159 patients. Patients characteristics are shown in (Tab.8).

Study group	Mean age	Sex		Smokers	Non smokers
		Female	Men		
159 patients	34,79	(73,6%)	(26,4%)	(16,4%)	(83,6%)

Table 8. The study patient groups’ characteristics

The men had higher level of Ig G antibodies than women see tab.9.

Group [U/ml]	Sex [U/ml]	
	Female	Men
study [1,83]	1,76	2,02

Table 9. The mean level of Ig G antibodies depending on the patient’s sex.

The majority of patients have moderate symptoms. Acute symptoms were only found in a minority of patients (Tab.10,11,12). The most common symptoms from the tested population are shown in the following tables.

Study group 159	Type of dyspeptic symptoms (%)			
	Severe	Moderate	Mild	Not specific
	18.2	59.8	17	5.0

Table 10. Severity of symptoms in the study population.

Study group (%)					
Upper abdominal pain	Heartburn	Discomfort in upper area	Belching	Nausea	Flatulence
76,1	62,2	68,5	50,3	32,7	59,8

Table 11. The types of symptoms in study group.

Study group			
Heartburn[%]	Frequency of symptoms [%]		
yes/not	Occasional	Once a week	Everyday
62,7/37,3	35,4	15,8	11,4

Table 12. The frequency of heartburn symptoms in the study group.

UBT showed that in the study of 81 (50.9%) dyspeptic patients, were infected with *H. pylori*, and ELISA tests were positive in 79 (49.7%) of patients. HelicoTest was positive in 88 (55.3%) of patients (Fig.6).

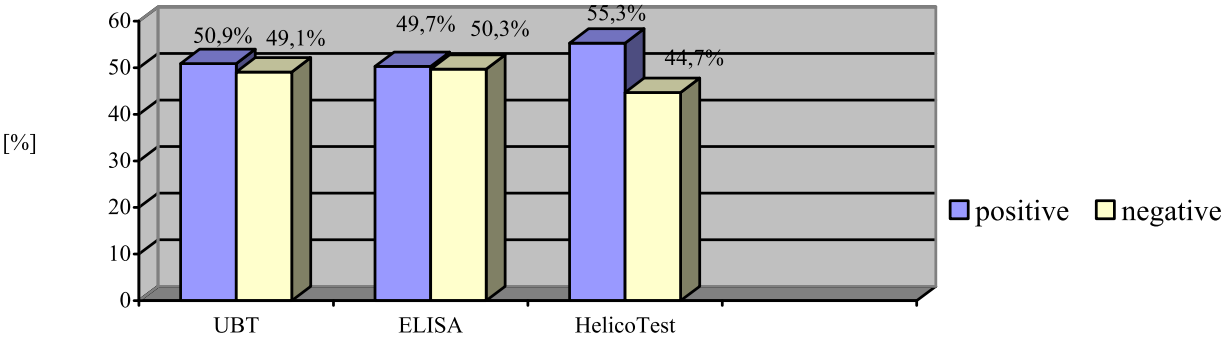


Fig. 6. The prevalence of *H. pylori* infection in the study group ($p>0,05$).

The increasing antibody level on ELISA showed the increasing probability of returning a positive UBT. For ELISA tests a positive result was defined as an antibody level of 1.12 (U/ml) using the Generalized Linear Model and 1.07 (U/ml) using the Generalized Additive Model. The IgG levels and the probability of *H. pylori* infection by UBT test is shown below (Fig.7).

The HelicoTest showed the highest prevalence of *H. pylori* infection, but the mean ELISA level by positive HelicoTest and negative UBT was 0.86 U/ml. The kappa coefficient for study group was 0.92 for UBT and ELISA and 0.66 for UBT and HelicoTest. The McNemary test showed no statistical differences in prevalence of *H. pylori* infection (Tab.13).

The majority of the young population tested had moderate dyspeptic symptoms. Analysis of the data showed little difference in detection of *H. pylori* among patients with acute, moderate, or mild dyspeptic symptoms, with patients with acute dyspeptic symptoms having the highest mean level of IgG (4.18 U/ml), (Fig.8).

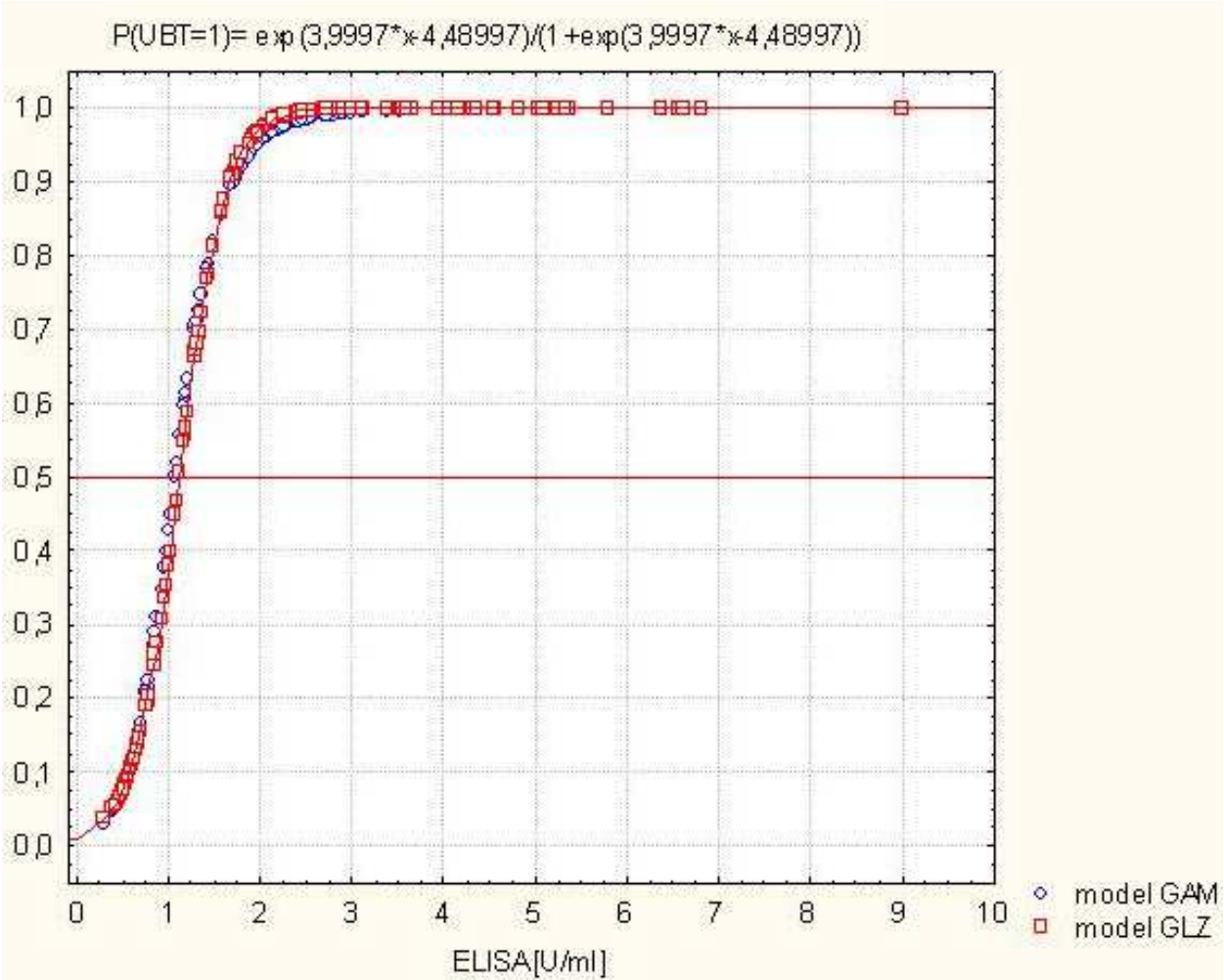


Fig. 7. The probability of *H. pylori* infection by UBT and IgG level in the study group by GAM and GLZ.

Tests	Study Group		
	n	x ²	p
UBT test and ELISA	159	0,29	0,593
UBT test and HelicoTest	159	2,33	0,157

Table 13. Test McNemary – for study group UBT, ELISA and HelicoTEST.

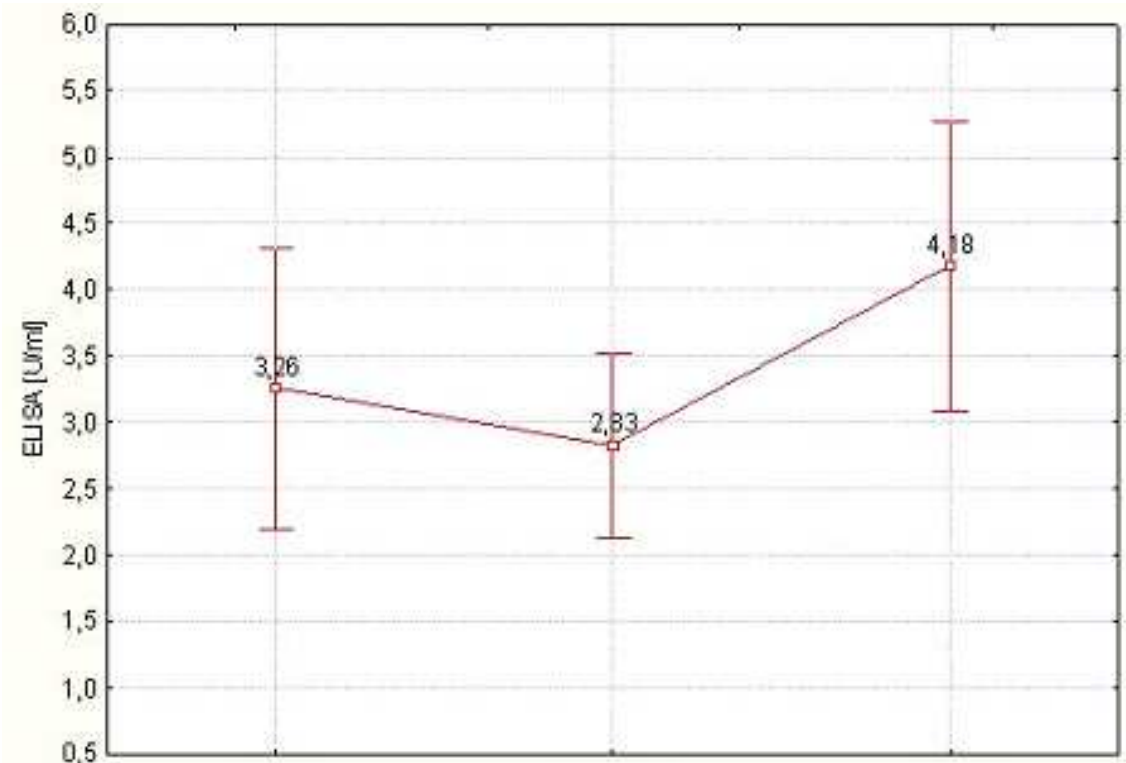


Fig. 8. The mean IgG level (U/ml) with mild, moderate and severe dyspeptic symptoms.

7. Discussion

Dyspepsia is an extremely common disorder affecting an estimated 40% of the world’s population. Only a minority of patients who experience symptoms will seek medical care, but dyspepsia still accounts for 5-7% of all visits to primary care physicians. Approximately 60% of young individuals reporting symptoms have FD, with the majority of these having no underlying organic cause of their symptoms. In countries with a high prevalence of *H. pylori* infection the “test and treat strategy” is a major way to manage undiagnosed dyspepsia. The economic models suggest that in populations of low *H. pylori* prevalence managing dyspepsia with empiric acid suppression therapy is a more cost-effective method.

Noninvasive testing for *H. pylori* infection is the main mode of testing for the young dyspeptic population. The correlation between the level of *H. pylori* IgG antibodies and UBT test is largely unknown as there is little research available which examines undiagnosed dyspepsia in patients under 45 years of age in a primary care setting. *H. pylori* infection is thought to be the most common factor for morbidity and mortality in upper digestive tract diseases.

We aimed to determinate the prevalence *H. pylori* infection and dyspeptic syndrome. In the USA patients under 55, and in Canada patients under 50, along with any patients suffering from dyspepsia without alarm symptoms should be tested for *H. pylori* infection and treated appropriately to control the symptoms (6,25). The optimal age threshold for endoscopy is unclear, and it is good to not that over half of the endoscopy results will not show any organic changes in standard endoscopy.

Research on high magnifying endoscopy and chromoendoscopy has showed that new methods are superior to standard endoscopy for diagnosis of *H. pylori* gastritis and finding some mucosal and capillary structures (34).

Noninvasive *H. pylori* testing is even more important now than in the past because dyspeptic problems are increasingly seen in the primary care setting, and diagnosis of *H. pylori* infection is important to the treatment of these patients. Past studies have shown that in a population with a high prevalence of *H. pylori* infection, the “test and treat” strategy is the best option (12). Overuse NSAID’s and in patients with risk factors, using cardioprotective doses of aspirin, eradication of *H. pylori* can be recommended.

The new classification of functional dyspepsia by Suzuki is based on a patient-centered approach based on the highest index of symptoms (24,28). We still do not know why the symptoms of dyspepsia fluctuate in the short-term. The strategy of rescreening *H. pylori* infection and eradication in high-risk population has already started (11,30,33).

The European Helicobacter Study Group (EHSG) was founded in 1987 to promote multidisciplinary research into the pathogenesis of *H. pylori* as a cause of upper gastrointestinal tract diseases, and how the treatment of *H. pylori* will aid in the prevention of gastric cancer (16). The EHSG has organized many annual consensus meetings and published current concepts regarding the management of *H. pylori* infection in Maastricht I, II, III and Maastricht IV (18,19,20).

The data in this study confirms that there is a correlation between the level of IgG antibodies and positive UBT test. There are some patients with dyspepsia and borderline results for *H. pylori* tests, and this is a problem that will require more research in the future (22). Right now, we strongly advise repeating the test in those patients whose symptoms persist because there is a correlation in the age and the prevalence of *H. pylori* infection in young dyspeptic patients and positive UBT test (21). There is a need to retest the population with dyspeptic symptoms and positive HelicoTest and negative UBT.

8. Conclusion

The level of IgG ELISA is a good predictor of probability of positive UBT. Serological testing by HelicoTest showed the highest prevalence *H. pylori* infection in young undiagnosed dyspeptic patients. Follow-up of these patients will be important to observe symptom relief and fluctuation in young dyspeptic patients.

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